

Mechanisms Underlying Sawtoothed Grain Beetle (*Oryzaephilus surinamensis* [L.]) (Coleoptera: Silvanidae) Infestation of Consumer Food Packaging Materials

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ABSTRACT The sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.), is an extremely destructive pest of packaged consumer food products. The beetle is not believed to chew directly through packaging materials, but to use openings or flaws in damaged or improperly sealed packages to gain entry. We investigated the behavioral mechanisms by which the sawtoothed grain beetle infests packages with flaws. Significantly more sawtoothed grain beetles infested consumer food packages that had been punctured with 0.4 mm diameter holes, to simulate packaging flaws that preclude adults, than when packages had no flaws. In a test arena, females laid more eggs into or near the hole in a plastic packaging film, when they were able to contact the food through the hole than when they could not contact the food. First instar larvae placed either 1 mm or 1 cm away entered holes when food was present, indicating that packages could become infested if eggs were laid near holes. In the absence of food, neither adults nor larvae responded to holes. This study has shown the importance of sound packaging in preventing insect infestation.

KEY WORDS behavior, food odors, oviposition, packaging

FOOD AND BEVERAGE packaging comprises over \$70 billion of the packaging market in the US and more than \$200 billion worldwide (Wilkinson 1998). The economic impact of insect infestation of packaged food on a company can be great. Consumers usually hold the manufacturer responsible for the insect infestation, regardless of where, when, or how the package became infested (Highland 1984). Food manufacturers know that if the consumer finds an insect in a cereal package, it can make a lasting negative and often irreversible impression, ultimately resulting in the loss of a customer. Little research has been conducted to describe the mechanisms by which insects enter packaged goods, but it is believed that most insects enter packages through existing openings.

The sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.), is a cosmopolitan invader of packaged consumer food (Highland 1991). Both larval and adult beetles are highly mobile. Packaging flaws may enable the beetles to enter, or in the case of females, to oviposit near or into packages where the larvae can then feed on the food product. Adults can fit through holes >0.71 mm diameter (Cline and Highland 1981) and larvae should be able to enter packages through extremely small openings. Mowery et al. (unpublished data) found that females responded to food odor em-

anating from holes in food packaging films by exhibiting area-concentrated search.

The objectives of this study were to (1) measure sawtoothed grain beetle infestation of consumer food if holes in packages are too small for adult entry; (2) determine female oviposition patterns in the vicinity of small holes in packaging materials that do not permit adult entry; and (3) assess the ability of neonates (newly hatched larvae) to locate existing holes or flaws in packaging to gain entry.

Materials and Methods

The sawtoothed grain beetles used in this study were obtained from lab-reared colonies maintained for over 15 yr at the USDA Grain Marketing & Production Research Center in Manhattan, Kansas (Kansas State University, Department of Entomology, voucher specimen number 132). The insects are maintained in climate-controlled incubators at a temperature of $27 \pm 2^\circ\text{C}$ and RH $50 \pm 5\%$. The beetles were reared on a diet consisting of rolled oats, flour, and brewer's yeast (Myers 1979).

Infestation of Consumer Food Packages. Insect-tight storage containers (clear polystyrene $41.2 \times 28.7 \times 25.3$ cm Rubbermaid Snap Toppers, Rubbermaid Inc., Wooster, OH) were used for the test, five for treatment food boxes with experimentally applied holes and five for controls without experimentally applied holes. Ten boxes of muffin mix (chiefly corn and wheat flour, frozen before test) were placed into

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each plastic storage container. The food boxes were $4 \times 9 \times 3.5$ -cm paperboard boxes with an inner polyethylene-coated kraft paper pouch. In treatment containers, the paper pouches were removed from all boxes, a single hole (0.4 ± 0.1 mm diameter, created using a #1 insect pin) was punctured into each of the four corners, pouches returned to boxes, and the boxes sealed shut using clear adhesive tape. The diameter of the holes allowed for the passage of eggs and young larvae but not adult beetles. Control boxes were not opened before testing.

One hundred beetles of mixed sexes were placed into each storage container, and the containers were then sealed shut using clear plastic packing tape to prevent insect escape. The storage containers were held under ambient conditions ($23.8 \pm 0.1^\circ\text{C}$ and $23.5 \pm 0.0\%$ RH). After 8 weeks, all of the food boxes were opened and the total numbers of adults and larvae inside the food pouches were counted and recorded.

Behavioral Tests

Test Arenas. Infestation behavior of both adults and neonates was assessed on 120 AB-X (acrylic on both sides, 1.2 mil gauge) packaging film (Anonymous 1995). Testing arenas were made by sandwiching a 5×5 cm piece of film between two 2.6 cm diameter polyvinyl chloride (PVC) plastic rings, which were ≈ 1.5 cm in depth. To prevent insect escape, 60×15 mm polystyrene plastic petri dishes (Becton Dickinson Labware, Franklin Lakes, NJ) were placed over each end of the arenas. A rubber band was stretched around the petri dish halves to hold the rings tightly together. Holes in the packaging film were created using a heated #1 insect pin (0.4 ± 0.01 mm diameter), which allowed passage of neonates but adult females were only able to fit their heads and ovipositors through holes. Each hole was positioned ≈ 0.3 cm from the arena edge.

Temperature, humidity, and light were controlled by running tests in humidity boxes held in an incubator. Arenas were placed in $26 \times 36.5 \times 15$ cm clear plastic boxes on top of two stacked plastic waffle grids (1.2 cm thick) with a saturated NaCl covering the lower grid to maintain humidity (Greenspan 1977). A HOBO data logger (BoxCar Pro, Onset Computer Corporation, Bourne, MA) was placed inside one of the humidity chambers. The average conditions were $27 \pm 0.01^\circ\text{C}$, $78 \pm 0.2\%$ RH, and tests were run in the dark.

Oviposition Test. Four treatments were compared to determine how contact with food influenced oviposition: (1) one 2 g piece of Butcher's Burger (Ralston Purina Co., St. Louis, MO) dog food (chiefly beef, soybean, and wheat, ground and compressed into pellets) placed on film surface and no hole in film; (2) one piece of food taped beneath the hole in the film, so that beetles are able to contact food through the hole; (3) one piece of food placed ≈ 1 cm beneath the packaging film, on the floor of the arena (i.e., beetles unable to directly contact the food); and (4) hole in film but no food. Before testing, 2-wk-old mated adult female beetles were held on dog food for 2 d. One female beetle

was added to each arena. Thirty replicates of each treatment were run, five replicates per humidity chamber. The arenas were incubated 6 d, after which time the number of eggs and their location within the arenas were recorded.

Neonate Test. To assess the ability of neonate larvae to find and enter holes, four treatments were tested using individual neonates: (1) one 2 g piece of Butcher's Burger dry dog food held against the underside of the hole with clear tape and the neonate was placed on the film surface 1 mm from the hole; (2) same as treatment 1, but the neonate was placed on the film 1 cm from the hole; (3) no food was present and the neonate was placed 1 mm from the hole; and (4) no food was present and the neonate was placed 1 cm from the hole. The packaging film and the inner surfaces of the arenas were sprayed with General Purpose Staticide (ACL Inc., Elk Grove Village, IL), to reduce neonate mortality because of static electricity present on the plastic rings. Before testing, sawtoothed grain beetle eggs were collected from the main colony, placed in 60×15 mm plastic petri dishes (Becton Dickinson Labware, Franklin Lakes, NJ) without food or water. Dishes were held in NaCl humidity chambers described above and neonates were collected after two days. The mean head capsule width of neonates was measured for 40 individuals and determined to be 0.27 ± 0.01 mm. Thus, neonates should be able to enter the 0.4 ± 0.01 mm diameter holes in the packaging film.

Insects were placed in the appropriate locations in the arenas using a paintbrush. Sixteen replicates were performed, four replicates per humidity box. The location of neonates (in hole or not in hole) was recorded twice in a 1-hr period.

Statistical Analysis. Two-tailed *t*-tests were performed using Statistix7 software (Analytical Software, Tallahassee, FL) or Microsoft Excel. To assess differences among treatments in the number of eggs laid, analyses of variance (ANOVA) and Tukey's studentized range test were used (SAS Institute 1987). The log-likelihood ratio tests for contingency tables (Zar 1999) were performed to identify differences in numbers of females that laid eggs and larvae that entered holes. Critical values were adjusted using the Bonferroni correction (Rice 1989) to account for multiple comparisons. Unless stated otherwise, data are presented as mean (\pm SEM).

Results

Infestation Test. Significantly more beetles were found in the treatment food boxes with experimentally applied holes (1479 ± 345 beetles, $n = 5$, per container of 10 boxes) than in control food boxes (186 ± 46 beetles, $n = 5$, per container of 10 boxes) ($t = 3.7$; $df = 8$; $P = 0.006$; two-tailed *t*-test for means). In treatment containers all the boxes were infested (10.0 ± 0.0) compared with an average of 2.6 ± 0.9 infested boxes for the control containers. The average number of insects per box, combining adults, larvae, and pupae, was 143 ± 16 ($n = 50$) for treatment boxes compared

Table 1. Oviposition (mean \pm SEM) by female sawtoothed grain beetles in an arena consisting of a plastic film with a 0.4 mm diameter hole placed in its surface held between 2.6 cm diameter PVC rings and with Butcher's Burger® dry dog food placed at different locations allowing different levels of contact

Treatment	Food Present	Contact with food	Number of females that laid at least one egg out of 30 tested	Number of eggs laid per female (mean \pm SEM)	Number of eggs laid per females that laid eggs (mean \pm SEM)
1	No	None	0	0 \pm 0	—
2	Yes	None	3	0.13 \pm 0.08	1.3 \pm 0.3
3	Yes	Partial	10	1.2 \pm 0.38	3.5 \pm 0.7
4	Yes	Full	29	10.7 \pm 1.11	11.0 \pm 1.1

with 52 ± 21 ($n = 12$) for control boxes. All infestations of the control boxes could be explained by pre-existing tears found in the packaging materials or improper sealing methods at the factory and represent real-life conditions in which infestations can occur.

Oviposition Test. There were differences in the number of females that oviposited in the different treatments (Table 1). More females oviposited when food was on top of the film than when food was taped under the hole, partially accessible ($G = 30.7$; $\chi^2_{0.016, 1} = 5.7$; $P < 0.02$), or when placed 1 cm below the film surface, not accessible ($G = 54.6$; $\chi^2_{0.016, 1} = 5.7$; $P < 0.02$). There was no significant difference in the number of females that oviposited when food was taped under the hole in the film than when food was 1 cm below the film (3 out of 30) ($G = 5.02$; $\chi^2_{0.016, 1} = 5.7$; $P > 0.02$). When food was placed on top of the film, eggs were typically adhered to the underside of the food or in among the frass. When food was placed 1 cm below the film, eggs were distributed over the film surface. All of the eggs found in the treatment in which the food was taped beneath the hole were laid through the hole in the film. Every female that oviposited chewed a depression into the dog food and laid their eggs through the hole, adhering them to the under-surface of the film.

There were also significant differences in the number of eggs laid among the treatments ANOVA, $F = 73.2$, $df = 2, 9$, $P = 0.01$. The treatment with a hole and no food was not included in analysis because no eggs were laid in any arenas. Female beetles deposited a greater number of eggs when the food was placed on top of the packaging film, as opposed to 1 cm beneath the film surface, or taped under the hole, ($P < 0.05$, Tukey's studentized range test) (Table 1). When only the females that actually oviposited are considered, there was a significant difference in the number of eggs laid between the treatment in which food was on top of the film and the treatment in which food was on the arena floor ($t = 3.96$, $df = 29$, $P = 0.0003$).

Neonate Test. More neonates entered the hole when food was present than when no food was present when starting at both 1 mm ($G = 24.3$; $\chi^2_{0.0125, 1} = 6.2$; $P < 0.02$) and 1 cm ($G = 16.7$; $\chi^2_{0.0125, 1} = 6.2$; $P < 0.02$) from the hole. When food was present 15 out of 16 larvae placed 1 mm from the hole entered within 30 min and all had entered within one hour, but only four out of 16 larvae, three in the first 30 min, entered the hole when no food was present. When food was present, 12 out of 16 larvae placed 1 cm away entered

the hole in the film within 30 min and one additional larva entered within 1 hr. When no food was present, 2 out of 16 larvae entered the holes; one larva went through the hole within a half-hour after the test began, and one larva within 1 hr. There was no difference in the number of neonates that entered the hole between the two distances when food was present ($G = 4.5$; $\chi^2_{0.0125, 1} = 6.2$; $P > 0.03$) or when it was not ($G = -1.2$; $\chi^2_{0.0125, 1} = 6.2$; $P > 0.03$).

Discussion

Finding resources involves a series of steps including habitat location, resource location, acceptance, and suitability (Laing 1937). Female sawtoothed grain beetles exploiting packaged food must locate flaws in the package. In some cases, these flaws may be large enough for them to enter the package. However, as the results from the infestation test with commercial packaged food indicate, sawtoothed grain beetle can infest packages through flaws that preclude adults from entering. When beetles are unable to enter the package, infestation may result from females laying eggs on the product through holes or eggs being laid outside and larvae crawling into packages. Results presented in this manuscript indicate that packages can be infested by both methods.

Previous studies have shown that female sawtoothed grain beetles do respond to food odors (White 1989, Trematerra et al. 2000) and exhibit localized search around packaging flaws that emit food volatiles (Mowery et al. unpublished data). In some cases volatile cues alone are sufficient stimulation to elicit oviposition, but results presented here indicate that contact with food is an important oviposition stimulant. As beetles were allowed more direct contact with food the number of females that laid eggs and the number of eggs laid increased. This indicates that a combination of tactile, gustatory, and olfactory cues may be involved in sawtoothed grain beetle oviposition.

The influence of the degree of food contact on oviposition suggests the role of multiple cues in oviposition. When food was 1 cm below the film (volatile cues only), few females deposited eggs and those that were laid appeared to be scattered across the film surface. When the food was taped to the underside of the hole (volatile cues and limited contact) all of the eggs were adhered to the underside of the film surrounding the hole. This was achieved through the ability of the female sawtoothed grain beetle to avert the mem-

branous ovipositor in a telescoping fashion (Mowery, personal communication). All females that oviposited in treatments with the food taped below the hole also chewed a depression into the dog food. When food was on top of the film, there was evidence that females chewed on the food and they tended to lay eggs under the food or in the frass material. These findings suggest that females prefer to oviposit into crevices (between the film and the food) and that feeding on the food, or at least in contact with the food, is an important oviposition stimulant. Chewing on food before oviposition may provide information about resource quality or it may be a method whereby the female manipulates the resource to facilitate neonate feeding.

Limited research has been conducted on the behavioral mechanisms used by stored product insects to infest packages. Barrer and Jay (1979) determined that the odor of kibbled wheat diffused through holes attracted gravid female free-flying almond moth, *Ephestia cautella* (Walker), and that they laid eggs in the vicinity of the holes. Volatile cues are important in the location of flaws for sawtoothed grain beetles because they arrest female movement, but the presence of volatile cues alone was not a strong stimulant to lay eggs near or in the package flaw. Mated female *Oryzaephilus surinamensis* have been shown to have a more rapid response to food odors than virgin females (White 1989).

Larvae emerging from eggs laid near food were able to locate the food source using volatile cues, so the infestation of packages by neonates hatching from eggs laid near holes is possible. This test has shown that larvae were attracted to the dog food odor when placed both 1 mm and 1 cm from the source, and not to the presence of holes in the surface of the films when no food odor was present. Similar behavioral responses to food odor have also been seen in neonate codling moth larvae, *Cydia pomonella* (L.) (Landolt et al. 1998). The larvae responded positively to the odor of immature apples in a Y-tube olfactometer, an enclosed arena bioassay, and in a straight tube olfactometer. Although females were less likely to lay eggs without food contact, entry of packages by neonates may still be important because females may lay eggs in food spillage in crevices around package flaws and the larvae may subsequently enter the package itself.

Hole size is important in sawtoothed grain beetle food package infestation. The range of hole sizes in commercial packages is large and difficult to measure. Holes larger than 0.71 mm diameter will allow adult entry (Cline and Highland 1981), and holes as small or possibly even smaller than 0.4 mm diameter where the food is accessible will allow females to lay eggs into the hole. Neonates can respond to food odors and crawl through holes as small as 0.27 mm diameter.

This research provides insight into the infestation behavior of one of the most destructive stored-product pests. It can aid in the development of improved packaging methods and materials. Further investigations into the infestation behavior of this and other stored-product insect species will be beneficial in

demonstrating to food manufacturers the importance of developing food packages of good insect resistance.

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